

WHAT IS CLAIMED IS:

1. A nucleic acid molecule comprising all or a portion of at least one viral genome and further comprising at least two recombination sites that do not substantially recombine with each other, wherein at least one recombination site is capable of undergoing recombination with a compatible recombination site in the presence of at least one protein active in lambda recombination and wherein the nucleic acid molecule replicates in prokaryotic and eukaryotic cells.
2. A nucleic acid molecule according to claim 1, wherein the nucleic acid molecule comprises all or a portion of at least one viral genome selected from the group consisting of an adenovirus genome and an adeno-associate virus genome.
3. A nucleic acid molecule according to claim 1, wherein the nucleic acid molecule comprises all or a portion of at least one retroviral genome.
4. A nucleic acid molecule according to claim 3, wherein the retroviral genome is a lentiviral genome.
5. A nucleic acid molecule according to claim 1, wherein the nucleic acid molecule comprises all or a portion of at least one viral genome selected from the group consisting of a herpesvirus genome and a pox virus genome.
6. A nucleic acid molecule according to claim 1, wherein the nucleic acid molecule comprises all or a portion of at least one RNA virus genome.
7. A nucleic acid molecule according to claim 6, wherein the RNA virus is selected from a group consisting of a flavivirus genome, a togavirus genome, and an alphavirus genome.
8. A nucleic acid molecule according to claim 1, wherein the nucleic acid molecule is a plasmid or a bacmid comprising a prokaryotic origin of replication and a selectable marker.

9. A nucleic acid molecule according to claim 1, further comprising one or more features selected from the group consisting of a promoter, a viral terminal repeat, a splice site, a packaging signal, a nucleic acid sequence responsive to one or more viral proteins, a recognition site, a recombination site, a sequences encoding one or more marker proteins or polypeptides, a sequence encoding one or more epitopes recognizable by an antibody, an origin of replication, an intervening sequence, an internal ribosome entry sequences, and a polyadenylation signal.

10. A nucleic acid molecule according to claim 1, further comprising a nucleotide sequence of interest between the two recombination sites.

11. A nucleic acid molecule according to claim 10, wherein the sequence of interest comprises one or more sequences selected from the group consisting a sequence encoding one or more polypeptides, a sequence encoding one or more tRNA sequences, a sequence encoding one or more ribozyme sequences, one or more promoter sequences, one or more enhancer sequences, and one or more repressor sequences.

12. A nucleic acid molecule comprising all or a portion of a baculoviral genome and further comprising one or more recombination sites, wherein at least one recombination site is capable of undergoing recombination with a compatible recombination site in the presence of at least one protein active in lambda recombination.

13. A nucleic acid molecule according to claim 12, wherein the molecule comprises two recombination sites that do not substantially recombine with each other.

14. A nucleic acid molecule according to claim 13, wherein the sequence of interest comprises one or more sequences selected from the group consisting a sequence encoding one or more polypeptides, a sequence encoding one or more tRNA sequences, a sequence encoding one or more ribozyme sequences, one or more promoter sequences, one or more enhancer sequences, and one or more repressor sequences.

15. A composition comprising the nucleic acid molecule of claim 1.

16. A composition comprising the nucleic acid molecule of claim 12.

17. A method of constructing a recombinant virus, comprising:
  - (a) providing a first nucleic acid molecule comprising all or a portion of at least one viral genome and at least a first and a second recombination site that do not substantially recombine with each other;
  - (b) contacting the first nucleic acid molecule with a second nucleic acid molecule comprising a sequence of interest flanked by at least a third and a fourth recombination site under conditions such that recombination occurs between the first and third recombination site and between the second and fourth recombination site; and
  - (c) introducing the nucleic acid molecule of step (b) into a cell that packages the nucleic acid molecule of step (b).
18. A method according to claim 17, wherein the first nucleic acid molecule comprises all or a portion of at least one viral genome selected from the group consisting of an adenovirus genome and an adeno-associate virus genome.
19. A method according to claim 17, wherein the first nucleic acid molecule comprises all or a portion of at least one retroviral genome.
20. A method according to claim 19, wherein the retroviral genome is a lentiviral genome.
21. A method according to claim 17, wherein the first nucleic acid molecule comprises all or a portion of at least one viral genome selected from the group consisting of a herpesvirus genome and a pox virus genome.
22. A method according to claim 17, wherein the first nucleic acid molecule comprises all or a portion of at least one RNA virus genome.
23. A method according to claim 22, wherein the RNA virus is selected from a group consisting of a flavivirus genome, a togavirus genome, and an alphavirus genome.
24. A method according to claim 17, wherein the first nucleic acid molecule is a plasmid or a bacmid comprising an origin of replication and a selectable marker.

25. A method according to claim 17, wherein the portion of the second nucleic acid between the recombination site comprises a nucleotide sequence of interest.

26. A method according to claim 25, wherein the sequence of interest comprises one or more sequences selected from a group consisting a sequence encoding one or more polypeptides, a sequence encoding one or more tRNA sequences, a sequence encoding one or more ribozyme sequences, one or more promoter sequences, one or more enhancer sequences, and one or more repressor sequences.

27. A method according to claim 17, further comprising digesting the first nucleic acid molecule with a restriction enzyme that cleaves the first nucleic acid at a site between the recombination sites.

28. A recombinant virus produced by the method of claim 17.

29. A composition comprising the recombinant virus of claim 28.

30. A method of producing a fusion polypeptide, comprising:  
providing a host cell comprising a first nucleic acid sequence encoding the fusion polypeptide, wherein the sequence comprises at least a first coding region and a second coding region separated by a sequence comprising a stop codon;

expressing in the cell a second nucleic acid sequence comprising one or more suppressor tRNAs that suppress the stop codon; and

incubating the host cell under conditions sufficient to express the fusion polypeptide comprising the first coding region and the second coding region, wherein at least one of the first and/or second nucleic acid sequences is present on a nucleic acid molecule comprising all or a portion of at least one viral genome.

31. A method according to claim 30, further comprising introducing nucleic acid molecules comprising the first and the second nucleic acid sequences into the host cell.

32. A method according to claim 30, wherein the nucleic acid molecule comprising the second nucleic sequence comprises all or a portion of an adenoviral genome.

33. A method according to claim 30, wherein the first coding region and stop codon are flanked by recombination sites that do not substantially recombine with each other.

34. A method of expressing a polypeptide, comprising:

contacting a cell with a nucleic acid molecule comprising a sequence encoding the polypeptide operably linked to a promoter and a repressor sequence, wherein the nucleic acid molecule comprises all or a portion of a viral genome;

contacting the cell with a nucleic acid molecule encoding a protein that binds to the repressor sequence; and

incubating the cell under conditions sufficient to express the polypeptide.

35. A method according to claim 34, wherein the viral genome is a lentivirus genome.

36. The method according to claim 34, wherein the viral genome is an HIV-1 genome.

37. The method according to claim 34, wherein the repressor sequence is the tetracycline operator sequence and the protein is the tetracycline repressor protein.

38. The method according to claim 37, wherein conditions sufficient to express the polypeptide comprise incubating the cell in the presence of tetracycline.

39. A method of expressing a polypeptide, comprising:

contacting a cell with a nucleic acid molecule comprising a sequence encoding the polypeptide operably linked to a promoter and a repressor sequence, wherein the nucleic acid molecule comprises all or a portion of a viral genome and wherein the cell expresses a protein that binds to the repressor sequence; and

incubating the cell under conditions sufficient to express the polypeptide.

40. The method according to claim 39, wherein the viral genome is a lentiviral genome.

41. The method according to claim 39, wherein the viral genome is an HIV-1 genome.

42. The method according to claim 39, wherein the repressor sequence is the tetracycline operator sequence and the protein is the tetracycline repressor protein.

43. The method according to claim 42, wherein conditions sufficient to express the polypeptide comprise incubating the cell in the presence of tetracycline.